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Evaluating damage in the perianth: a new diagrammatic scale to estimate population level of *Aceria guerreronis* Keifer (Acari: Eriophyidae) in coconut fruits

Avaliação do dano no perianto: uma nova escala diagramática para estimar o nível populacional de *Aceria guerreronis* Keifer (Acari: Eriophyidae) em frutos de coqueiro

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Abstract

The coconut mite, *Aceria guerreronis* Keifer (Acari: Eriophyidae), is one of the main pests of the coconut crop by attacking the fruits. Colonies can reach 10,000 mites/fruit at high infestation. A diagrammatic scale (Galvão scale) has been developed and tested by Brazilian researchers to estimate the number of mites/fruit by determining the percentage of necrotic area of the fruit surface. Our objective was to develop and validate a new diagrammatic scale based on the percentage of the damaged perimeter of the fruit at the base of the perianth, in order to facilitate and improve the quantification of *A. guerreronis* per fruit. Fruits were collected in an urban coconut plantation in Ilhéus, Bahia, Brazil. For the development of the new diagrammatic scale, it was calculated the percentage of the damaged perimeter on 50 fruits with different damage levels. To analyze the relationship between the damage and population levels of *A. guerreronis* on the fruits, we compared the two scales by testing them both on 470 fruits. The new diagrammatic scale recorded nine damage levels, 1, 2, 4, 8, 16, 32, 48, 70 and 100%. To validate our new diagrammatic scale, the damage level in each fruit was estimated by 10 inexperienced evaluators, with and without the aid of the scale. The accuracy and precision of each evaluator were determined through linear regression between observed and estimated damage. With or without or the use of the scale, 5 of 10 evaluators overestimated or underestimated the damage level and were imprecise and inaccurate. This showed that the scale did not provide improvements in the levels of accuracy and precision of the evaluators. The relationship between infestation and damage levels showed high accuracy for both scales in the regression analysis (R^2= 96% and R^2= 98%). The population peak of *A. guerreronis* by the new diagrammatic scale occurred at 32% of damage level and for the Galvão scale it occurred at 8% of damage level. Although the new diagrammatic scale has not promoted improvements in damage estimates, it presented high precision in the relationship between infestation and damage levels.

Key words: Acari, coconut mite, *Cocos nucifera*, eriophyid, scale of damage.

Resumo

O ácaro-da-necrose-coqueiro, *Aceria guerreronis* Keifer (Acari: Eriophyidae), é considerado uma das principais pragas da cultura, por atacar os frutos. Em altas infestações, as colônias podem chegar a 10.000 ácaros/fruto. Uma escala diagramática (escala de Galvão) já foi desenvolvida e testada por pesquisadores brasileiros para estimar o número de ácaros/fruto através da determinação da porcentagem da área necrosada da superfície do fruto. Nosso objetivo foi desenvolver e validar uma nova escala diagramática, baseada na porcentagem do perímetro do fruto danificado na base do perianto, para facilitar e melhorar a quantificação do número de *A. guerreronis* por fruto.

Os frutos foram coletados em um plantio urbano de coqueiros em Ilhéus, Bahia, Brasil. Para o desenvolvimento da nova escala diagramática, foi calculada a porcentagem do perímetro danificado em 50 frutos com diferentes níveis de dano. Para analisar a relação entre os danos e os níveis populacionais de *A. guerreronis* nos frutos, as duas escalas foram comparadas, aplicando-se ambas em 470 frutos. A nova escala diagramática registrou nove percentuais de dano, 1, 2, 4, 8, 16, 32, 48, 70 e 100%. Para validar nossa nova escala diagramática, o nível de dano em cada fruto foi estimado por 10 avaliadores inexperientes, sem e com o auxílio da escala. A acurácia e a precisão de cada avaliador foram determinadas por regressão linear entre o dano observado e estimado. Sem e com a utilização da escala, 5 dos 10 avaliadores superestimaram ou subestimaram o nível de dano e ainda foram pouco precisos e inexatos, demonstrando que a escala não propiciou melhorias nos níveis de acurácia e precisão dos avaliadores. A relação entre infestação e níveis de dano apresentou elevada precisão para as duas escalas nas análises de regressão (R^2= 96% e R^2= 98%). O pico populacional de *A. guerreronis* pela nova escala diagramática ocorreu no nível de dano de 32% e pela escala de Galvão ocorreu no nível de dano de 8%. Embora a nova escala diagramática não tenha promovido uma melhoria nas estimativas de dano por avaliadores inexperientes, a mesma apresentou elevada precisão na relação entre infestação e níveis de dano.

Palavras chave: Acari, ácaro-do-coqueiro, *Cocos nucifera*, eriofiídeo, escala de dano.

Introduction

Aceria guerreronis Keifer (Acari: Eriophyidae) colonizes coconut fruits and is considered one of the main pests of coconut in the Americas, Africa and parts of Asia (Navia, Moraes, Lofego, & Flechtmann, 2005; Navia, Gondim, Aratchige, & Moraes, 2013; Aratchige, Sabelis, & Lesna, 2007; Galvão, Gondim, & Michereff, 2008; Negloh, Hanna, & Schausberger, 2010), causing fruit drop and/or reduced weight/size, albumen volume and decreased commercial value of the fruits (Galvão, Gondim, Moraes, & Melo, 2011). Their colonies can reach 10.000 mites per fruit, feeding on the meristematic region underneath the bracts (perianth) (Lawson-Balagbo, Gondim, Moraes, Hanna, & Schausberger, 2007; Souza, Gondim, Ramos, Santos, Ferraz, & Oliveira, 2012). The initial damage may be detected as a small triangular chlorotic area on the fruit epidermis exposed close to the distal margin of the inner bracts, which gradually increases and becomes larger and necrotic, inducing longitudinal cracks as fruit develops (Howard & Moore, 2006; Aratchige *et al.,* 2007).

The control of *A. guerreronis* has been mainly based on acaricides, although other strategies have been investigated, such as the elimination of infested fruits and biological control with predators and pathogens (Domingos et al., 2010; Lima, Melo, Gondim, & Moraes, 2012; Lima, Guedes, Siqueira, Pallini, & Gondim, 2013; Melo, Domingos, Pallini, Oliveira, & Gondim, 2012). Regardless of the control strategy, the determination of the population level of *A. guerreronis* is fundamental in integrated mite management programs, which require accurate and practical tools to estimate infestation.

Negloh, Hanna, & Schausberger, (2011), classified fruits into different damage categories based on the percentage of visible damage in relation to undamaged surface area to estimate the average population level of *A. guerreronis* in each category. Galvão *et al.,* (2008), developed a diagrammatic scale (Galvão scale) with damage levels varying from 1 to 70% to standardize the estimation of the percentage of visible damaged in relation to the total area of the fruits and related those damage levels with the population levels of *A. guerreronis* in 2 to 6 months-old fruits. The Galvão scale was applied by Souza *et al.,* (2012), to classify the damage levels in 4 months-old fruits to estimate the average population levels of *A. guerreronis* in each category. Although the Galvão scale added accuracy and precision to the visual analysis of the percentage of damage, in the other hands, it presents two difficulties: (1) it is necessary to evaluate the damages in the different fruit faces to classified the damage level for each fruit, and (2) the evaluated damages were mainly caused in the past, not representing recent infestation on the perianth.

The aim of the present research was to develop and validate a new diagrammatic scale based on the percentage of damaged fruit epidermis close to the perianth in order to improve the estimation of the damage and the population levels of *A. guerreronis* in comparison to the Galvão scale.

Material and methods

Sample of fruits

Coconut fruits from 1 to 5 months-old bunches with different damage levels by *A. guerreronis* were collected in an urban coconut plantation containing the green dwarf variety located in the municipality of Ilhéus, in the State of Bahia, northeastern Brazil (14°48′41″S, 39°2′20″W). The fruits were stored in polyethylene bags, transported to the laboratory and stored in a refrigerator at 15 °C for up to 10 days until the evaluations. The storage of the fruits was necessary to maintaining the mite population levels stabilized during the several days needed to finalize evaluations on all the fruits.

Development and validation of the new diagrammatic scale

The total and damaged perimeter close to the perianth of 50 fruits was measured with a millimeter tape at the level of the distal margin of the inner bracts for determination of the percentage of damage for each fruit (Figure 1). A new diagrammatic scale with geometric damage levels was developed based on the maximum and minimum damage levels observed. The damage level in each fruit with and without the aid of the new diagrammatic scale was estimated by 10 inexperienced evaluators. A second evaluation with the aid of the new diagrammatic scale by the same evaluators was carried out seven days later to evaluate the repeatability.

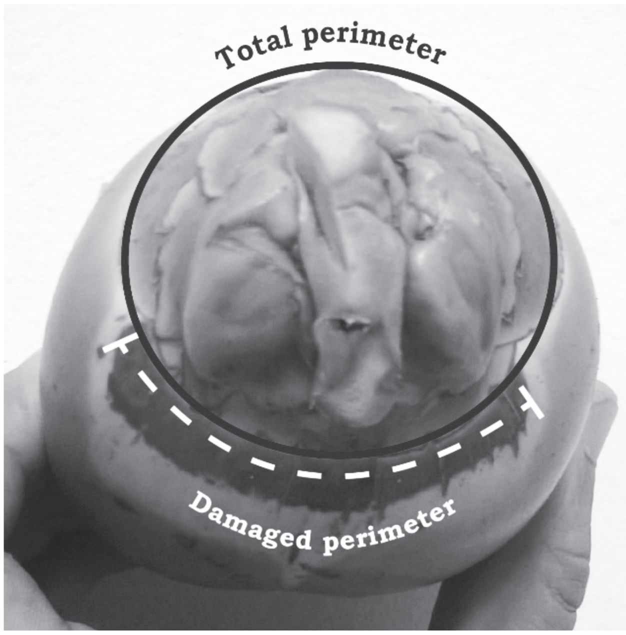


Figure 1. Measurements taken on the coconut fruits: the total and damaged perimeter around the bract

The accuracy and precision were determined for each evaluator through linear regression analysis, considering the real damage as an independent variable and the estimated damage as a dependent variable. The accuracy of the estimates for each evaluator, as well as for the group of evaluators, was determined by a *t*-test applied to the intercept of linear regression (*a*), to verify the hypothesis Ho: $a = 0$, and the slope of the straight line (*b*), to test the hypothesis Ho: $b = 1$ ($P = 0.05$). Intercept values significantly different from zero indicate overestimation (>0) or underestimation (<0) of the real damage at low damage levels. Slope values significantly different from one indicate overestimation (>1) or underestimation (<1) of the real damage at all damage levels. The precision of the estimates was determined by the coefficient of determination of the linear regression (R^2), by the variance of the absolute errors (estimated damage - real damage) and by the repeatability of the estimates, determined by the regression of the second evaluation compared to the first evaluation in the same sample unit. The regression analyses were accomplished using SigmaPlot® v12.0 and TableCurve® 2D v5.01 (Systat, 2002) programs.

Quantification of *A. guerreronis* in relation to the damage levels

The new diagrammatic scale and the scale based on the estimation of the percentage of visible damaged in relation to the total area of the fruits (Galvão *et al.,* 2008) were applied to 470 fruits. The bracts were removed and the *A. guerreronis* mites present in the inner and outer surfaces of the bracts of each fruit, as well as the in the fruit surface under the bracts, were transferred using a wash bottle and a brush to a 140 mL vial with 70% alcohol and one drop of surfactant Tween® 20. After deposition of *A. guerreronis* in the bottom of the vial, the volume of liquid was adjusted to 20 ml. It was agitated slightly during 30 s with a Pasteur pipette and transferred (3 mL sample) to an acrylic box. The number of *A. guerreronis* per fruit was counted and estimated under a microscope (Galvão *et al., 2008*; Reis *et al.,* 2008; Souza *et al., 2012*). Linear and non-linear regression analysis were carried out to select the models with the best fit of the relationship between the percentage of damaged perimeter and the number of mites per fruit, based on R^2 and on the residual mean square (RMS). Significance of the regressions was verified by *F*-test. All regression analyzes were performed using SigmaPlot® v12.0 and TableCurve® 2D v5.01 programs (Systat, 2002).

Results and discussion

Development and validation of the new diagrammatic scale

The new diagrammatic scale registered the damage levels of 1, 2, 4, 8, 16, 32, 48, 70 and 100% (Figure 2).

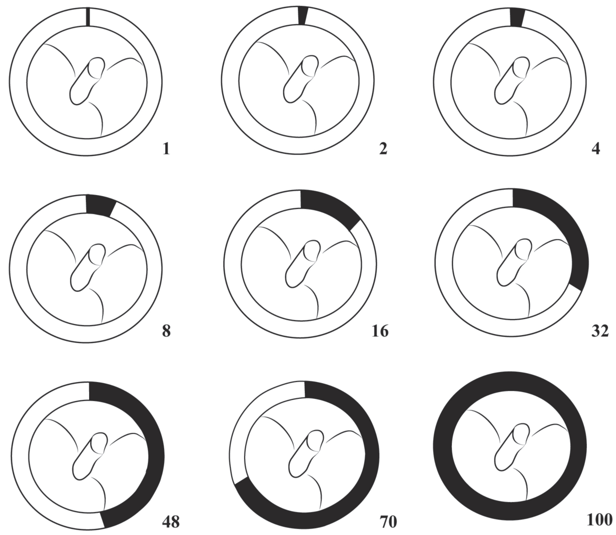
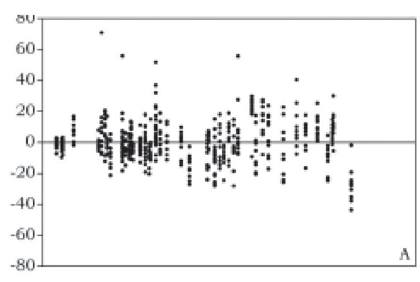


Figure 2. New diagrammatic scale based on the percentage of damaged perimeter (black) at the level of the distal margin of the inner bracts in coconut fruits infested by *A. guerreronis* indicating the damage levels of 1, 2, 4, 8, 16, 32, 48, 70 and 100%.

Without the aid of the new diagrammatic scale, *a* values were significantly higher than 0 for two evaluators out of 10, *b* values were significantly different from 1 for one evaluator, and R^2 values ranged from 0.49 to 0.82, with an average of 0.72 (Table 1). Estimations of the absolute errors (estimated damage less real damage) ranged from -43.5 to 70.9 (Figure 3A). With the aid of the new diagrammatic scale in the first evaluation, *a* values were significantly higher than 0 for two evaluators out of 10, *b* values were significantly different from 1 for two evaluators, and R^2 values ranged from 0.58 to 0.84, with an average of 0.75 (Table 1). Estimations of the absolute errors (estimated damage less real damage) ranged from -48.7 to 37.7 (Figure 3B). In the second evaluation, *a* values were significantly higher than 0 for five evaluators out of 10, *b* values were significantly different from 1 for seven evaluators, and R^2 values ranged from 0.59 to 0.82, with an average of 0.76 (Table 1). Estimations of the absolute errors (estimated damage less real damage) ranged from -42.4 to 48.8 (Figure 3C).



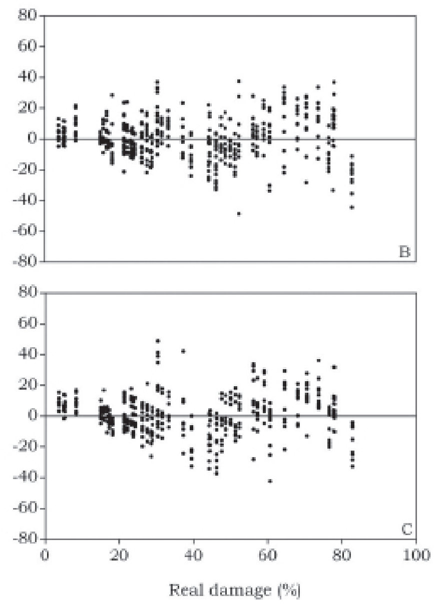


Figure 3. Distribution of absolute errors (estimated damage – actual damage) of estimates of damage of *A. guerreronis* in coconut fruits assessed without (A) and with the aid of the new scale in the first (B) and second evaluation (C) in 10 evaluators.

Table 1. Intercept (*a*), slope of the straight line (*b*) and coefficient of determination (R^2) of linear regression equations relating visual estimates of damage of *A. guerreronis* in coconut fruits performed by evaluators with and without the diagrammatic scale

Evaluators	Without scale			With scale					
				1st Evaluation			2nd Evaluation		
	a	b	R^2	a	b	R^2	*a*	*b*	R^2
A	-5,23	0,96	0,53	-9,27	0,93	0,58	-6,25	0,95	0,59
B	-0,86	0,93	0,68	-15,19*	1,11	0,70	-16,07*	1,08	0,68
C	0,38	1,10	0,80	-6,60	1,15	0,76	-7,17*	1,23*	0,82
D	3,57	1,17*	0,81	-4,74	1,42*	0,84	-9,86*	1,39*	0,77
E	1,07	1,09	0,70	1,68	1,18	0,74	-7,87	1,35*	0,76
F	0,67	1,15	0,80	-2,21	1,20*	0,83	-3,87	1,35*	0,82
G	16,51*	0,87	0,49	1,65	1,07	0,61	-9,89*	1,40*	0,76
H	8,36*	1,07	0,80	3,63	1,09	0,81	-10,42*	1,33*	0,78
I	4,06	1,01	0,82	-7,11*	1,13	0,81	-1,69	1,06	0,81
J	2,70	1,11	0,79	-2,20	1,13	0,76	-7,72	1,23*	0,79
Mean	-	-	**0,72**	-	-	**0,75**	-	-	0,76

* Null hypothesis (*a* = 0 ou *b* = 1) rejected by the test *t* (*P* = 0.05).

When comparing the second with the first evaluation of damage using the new diagrammatic scale, the evaluators showed a high coefficient of repeatability, with a mean of 80% (R^2). Between the evaluations, *a* values were significantly higher than 0 for three evaluators out of 10, *b* values were significantly different from 1 for only one evaluator (Table 2).

Table 2. Intercept (*a*), slope of the straight line (*b*) and coefficient of determination (R^2) of linear regression equations relating the second with the first evaluation of the damage of *A. guerreronis* in coconut fruits performed by the same evaluator with the new diagrammatic scale

Evaluators	a	b	R²
A	1,24	0,87	0,79
B	5,90*	0,92	0,82
C	4,78	0,94	0,83
D	-1,47	0,90	0,78
E	-4,36	1,02	0,82
F	1,42	1,05	0,87
G	3,77	0,93	0,63
H	-9,48*	1,10	0,78
I	7,92*	0,85*	0,82
J	-0,58	0,97	0,82
Mean	-	-	**0,80**

* Null hypothesis (*a* = 0 ou *b* = 1) rejected by the test *t* (P = 0.05).

The reproducibility, represented by the frequency distributions of the R^2 values, was high between the evaluators with and without the use of the new diagrammatic scale, because in 70% and 80% of the cases, respectively, it ranged from 0.70 and 0.89 (Table 3).

Table 3. Frequency distributions of the coefficient of determination (R^2) (%) of linear regression equations relating the estimates of damage of *A. guerreronis* between the evaluators, without and with the new diagrammatic scale in the first and second evaluations

Intervals of the coefficient of determination (R²)	Frequency (%)		
	Without scale	With scale	
		1st Evaluation	2nd Evaluation
0,40 – 0,49	10,00	0,00	0,00
0,50 – 0,69	20,00	20,00	20,00
0,70 – 0,89	70,00	80,00	80,00
0,90 – 1,00	0,00	0,00	0,00

Quantification of *A. guerreronis* in relation to the damage levels

With the new diagrammatic scale, the logarithmic model $y = 159565,7641/x^{[-0,5(\ln(x/54,2589)/0,6485]^2}$ provided the data fitting with high precision (R^2 = 0.9564 and P<0.0001), being y the mean number of mites per fruit and x the estimated damage level. The mean number of *A. guerreronis* in the fitted model increased from 30 to 3,600 mites/fruit with damage levels ranging from 1 to 32%; it declined until reaching to 1,000 mites/fruit in 100% of damage level. With Galvão scale, the logarithmic model $y = 43850.9449/x^{[-0,5(\ln(x/16,9814)/0,8536]^2}$, also provided the data fitting with high precision (R^2 = 0.9778 and P<0.0001), being y the mean number of mites/fruit and x the estimated damage level. The mean number of *A. guerreronis* increased from 180 to 3,700 mites/fruit, with damage levels ranging from 1 to 8%, declining until reaching to 160 mites/fruit in 70% of damage level (Figure 4).

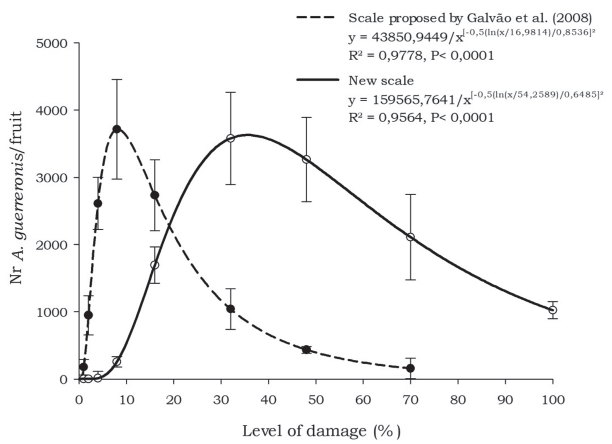


Figure 4. Regression models for the relationship of *A. guerreronis* population levels to the damage levels in coconut fruits assessed by the new diagrammatic scale and Galvão scale.

The use of the new diagrammatic scale of damage at the level of the distal margin of the inner bracts did not improve the accuracy and precision of the evaluators to estimate the damage in coconut fruits. Without the aid of the new diagrammatic scale, a trend towards overestimation of the damage was observed. In contrast, with the aid of the new diagrammatic scale we observed a trend to underestimate the damage for a few evaluators, and a non-improvement in the accuracy and precision of the estimates in the first evaluation. Using the new diagrammatic scale in the second evaluation a reduction in the variations of absolute errors was observed, which in the residue analysis corresponded to the difference between the estimated damage values and the actual damage. Furthermore, a decline in the accuracy to more evaluators was noted in the second evaluation, despite the training and accustoming to using. Our results differ from those reported by Nutter, Esker, & Coelho Netto (2006); Galvão *et al.,* (2008), who recorded an improvement in the estimates through the training of evaluators in evaluations of plant diseases as well as the necrotic area of the coconut fruit surface.

Although the use of the new diagrammatic scale has promoted a little reduction in the variation of the errors, a good repeatability and a slight improvement in the reproducibility, in general the results were not good enough. However, the new diagrammatic scale proposed shows a quantitative promise as an estimating tool of damage in coconut fruits; but it must be improved and also tested under field conditions by farmers who have more visual expertise with this kind of damage.

The analysis of the percentage of necrotic area of the fruits has been used as the main estimating tool for evaluation of damage on coconut fruits (Negloh *et al., 2011*; Galvão *et al.,* 2011). However, this type of analysis shows two problems: (1) the needs for partial evaluations on the three sides of the fruit, which makes it less useful because it increases the probability of error, and (2) because it estimates the past damage caused by *A. guerreronis* populations. The proposed evaluation of the damage on the perimeter comes to solve both problems, because (1) it evaluates the damage on the top of the region of the perianth, and not on the sides; it allows visualization of the distal margin of the inner bracts, and so an estimate based on a single and uniform measure; and (2) it estimates the damage caused by the present populations of *A. guerreronis* since the analysis is based on the percentage of the chlorotic perimeter, which expands at the distal margin of the inner bracts under where these eriophyid mites inhabit.

The results showed a variation in the relationship between the population levels of *A. guerreronis* in function of the damage levels in coconut fruits, either by the application of the new diagrammatic scale, as well as by the Galvão scale. With the application of both scales, the populations of *A. guerreronis* were low at inferior damage levels, reached a peak at intermediate levels, and declined at high damage levels. It has been observed in several studies and seems to represent a pattern (Negloh *et al., 2011*; Galvão *et al.,* 2011). The highest population levels was recorded between 32 to 48% damage levels when applying the new diagrammatic scale; when the Galvão scale was used this occurred between 4 and 16%. Differences in the mite population levels between these scales can be explained by the distinct parameters of damage evaluation.

Evaluations conducted in Brazil with the aid of the Galvão scale, suggest that the relationship between the damage level and the population peak of *A. guerreonis* probably varies according to locality, climate, biotic and abiotic factors, variety, and age of fruits. We found that the population peak occurred at 8% of damage level in coconuts fruits (green dwarf) with 1-5 months-old. In the State of Alagoas, a population peak of *A. guerreronis* was reported at 16% of damage level in green dwarf coconut fruits with 2-6 months-old (Galvão *et al.,* 2008). In Bahia, this peak was observed in coconuts (green dwarf x GOA) with 4 months-old at 32-48% of damage level (Souza *et al.,* 2012).

The pattern of variation of *A. guerreronis* population level suggests that the dispersal of these mites can occur after they reach high densities, probably due to intraspecific competition caused by scarcity of food and space, interspecific competition with other mites such as *Steneotarsonemus* spp., and/or to predators such as *Neoseiulus* spp. (Negloh *et al.,* 2011; Galvão, Melo, Lima, Moraes, & Gondim, 2012). Furthermore, it is possible that the increasing of lignin content in the meristematic tissue of the fruit, and also the injection of toxins released with the saliva during plant feeding, may cause the decline of *A. guerreronis* populations due to their movement to feed on new cells (Galvão *et al.,* 2008; Reis, Gondim, Moraes, Hanna, Schausberger, Lawson-Balagbo, & Barros, 2008; Souza *et al.,* 2012). Another possibility is that after reaching the population peak with increased necrosis the mites tend to disperse by wind. This is a fact that seems to be common in environments with availability of food resources that can be influenced by biotic and abiotic factors (Melo *et al.,* 2012; Melo, Lima, Sabelis, Pallini, & Gondim, 2014).

Although the new diagrammatic scale proposed in this study did not result in an improvement in the estimative of damage in coconut fruits, in the other hands, it makes possible to correlate the population levels of *A. guerreronis* on the fruit with the damage levels pre-established for the scale with yielding consistent results. Thus, the damage levels predetermined in this work may facilitate the determination of the population level of *A. guerreronis* in integrated management programs, and can also be useful for future studies to help the understanding of various ecological and behavioral processes of this eriophyid mite, achieving best quality of *A. guerreronis* population estimates.

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